ISSN: 2755-0184

Journal of Diabetes Research Reviews & Reports

SCIENTIFIC Research and Community

Research Article

Open Access

Isopropyl Nitrite Induced Hemoglobin Oxidation in Diabetics Blood

John Philip Tarburton

Associate Professor Department of Mathematics and Natural Sciences, National University Los Angeles, USA

ABSTRACT

The effect of isopropyl nitrite on human Type 2 Diabetes blood was undertaken using non diabetics blood as the control group. The differences in patient characteristics such as the mean ages and weights of the two groups were not statistically significant (P>0.05), and the ratios of non-smokers to smokers were similar meaning that the two groups were well matched. These studies revealed that diabetics erythrocytes with a mean HbA1C value ± SEM of 11.4 ± 0.27% were oxidized at a significantly greater rate than that of the control blood (P<0.05). The isopropyl nitrite mean oxidation time ± SEM of diabetics blood was 1.5 ± 0.05 min (n = 20). For the nondiabetics blood a mean HbA1C ± SEM value of 5.5 ± 0.08% was obtained with a mean oxidation time ± SEM of the non-diabetics blood of 4.6 ± 0.13 min (n=20). These studies demonstrate that Diabetes blood has an enhanced susceptibility of oxidation into methemoglobin by isopropyl nitrite compared to its respective control group, i.e., the normal blood. This finding could be attributed to the fact that isopropyl nitrite is a nitrite ester which contains a saturated three hydrocarbon chain similar to other analogous nitrite esters (ethyl nitrite, butyl nitrite, pentyl nitrite and hexyl nitrite) which also contain saturated hydrocarbon chains that previously showed a statistically significant increased oxidation time for diabetics blood (P<0.05) [1-6]. Thus this study confirms that the difference in the number of methylene molecules has no impact on the rate of oxidation on either diabetics blood or nondiabetics blood (P>0.05). These findings also imply that the increased susceptibility to isopropyl nitrite induced oxidation reaction in diabetics blood is a direct function of the amount of HbA1C present in the blood, i.e., a clear inverse relation appears to exist between the amount of HbA1C present and the oxidation time.

***Corresponding author**

John Philip Tarburton, Associate Professor Department of Mathematics and Natural Sciences, National University Los Angeles, USA, E-Mail: jtarburton@nu.edu

Received: Apr 02, 2020; **Accepted:** May 03, 2020; **Published:** May 13, 2020

Keywords: Isopropyl Nitrite, Hba1c, Methemoglobin, Type 2 Diabetes Mellitus

Introduction

Isopropyl nitrite belongs to a class of compounds called alkyl nitrites or nitrite esters that cause oxyhemoglobin to undergo oxidation, i.e. the iron (II) in the hemoglobin loses an electron to become iron (III) and cannot carry oxygen to the tissues and is therefore useless in oxygen transport to the tissues. Nitrites are compounds that have long been known to induce this oxidation reaction. Any number of nitrite esters such as ethyl nitrite, isopropyl nitrite, butyl nitrite, pentyl nitrite and hexyl nitrite are used as inhalants known as poppers. They are easily obtained and overuse can cause side effects such as tachycardia, migraine headaches, fainting and dizziness. Isopropyl nitrite poppers can cause Maculopathy. Collectively, it has been discovered that alkyl nitrites may induce neurotoxicity which mainly impacts learning and memory function. If swallowed poppers can cause cyanosis followed by death due to too much methemoglobin because the hemoglobin can no longer transport the amount of oxygen required by the tissues which is a condition referred to as acquired Methemoglobinemia. Because of the wide usage of these nitrite esters especially isopropyl nitrite for recreational consumption coupled with side effects arising from their use a comparative study of Diabetes blood vs. normal blood appears warranted [7-18].

The goal of these studies is to help establish that people with type 2 diabetes mellitus have a greater risk of enhanced methemoglobin formation by alkyl nitrites compared with normal (nondiabetics) blood. To this end statistical methods can be employed to identify the 'at risk' group, e.g., diabetics, by using Student's t-test. This could later be followed by the requisite ad hoc statistical tests including linear regression analysis and the coefficient of determination calculation provided the Diabetes blood first showed an enhanced susceptibility to oxidation by isopropyl nitrite. All of this would then provide supportive reasons to indicate that alkyl nitrite use in any form should be avoided for diabetics. In this particular study isopropyl nitrite will be investigated to see how its properties correspond to other alkyl nitrites that have been previously investigated so as to obtain a more comprehensive understanding of the enhanced susceptibility of Type 2 Diabetes blood to alkyl nitrites.

Materials and Methods

Isopropyl nitrite was purchased from Win-Win Chemical CO, Limited. Other required chemicals were obtained from the Sigma and Aldrich Chemical Company. Blood products such as normal adult blood and Diabetes blood were purchased from Physicians Plasma Alliance (PPA). All subjects who participated in the studies gave voluntary informed consent. PPA identified the source of Diabetes blood as being from patients with type 2 diabetes mellitus. The procedures followed by PPA for this sample

collection study were in accordance with the ethical standards of the Hummingbird IRB Protocol wherein all subjects used in these studies gave voluntary informed consent. All blood was tested and certified to be non-viral by PPA. For these studies the data was obtained from 40 donors 20 of whom had type 2 diabetes mellitus and 20 of whom were nondiabetics. In Tables 1 and 2 the characteristics of the patients used in these studies are presented, i.e., HbA1C percentage, age, gender, weight, smoker status and oxidation times (in min). All blood was drawn into ACD tubes and stored at 2-4 C prior to use. The Hemoglobin A1C (HbA1C) percentages were determined using a Bayer DCA-2000 test kit. Diabetes was assessed as a HbA1C percentage greater than 6.5% [19].

Table 1. Characteristics of Diabetic Patients in the Isopropyl Nitrite Oxidation Studies

Sample ID	HbA1C (%)	Age (yrs)	Gender	Weight (lbs)	Smoker status	Oxidation Time (min)
GWB005533	13.3	50	Male	167	Smoker	1.2
GWB005544	11.4	43	Female	254	Non-Smoker	1.5
GWB005547	10.4	60	Female	223	Non-Smoker	1.7
GWB005548	10.6	48	Female	167	Non-Smoker	1.7
GWB005549	12.7	21	Female	282	Smoker	1.2
GWB005550	10.9	21	Male	158	Non-Smoker	1.5
GWB005551	11.1	46	Male	252	Non-Smoker	1.3
GWB005553	10	57	Male	207	Non-Smoker	1.8
GWB005556	13.7	41	Female	300	Smoker	1.2
GWB005564	10.1	47	Female	219	Non-Smoker	1.7
GWB005694	11.8	41	Male	255	Non-Smoker	1.5
GWB005690	10.2	45	Male	330	Non-Smoker	1.8
GWB005675	11.7	41	Male	314	Non-Smoker	1.5
GWB005683	10.7	27	Male	245	Non-Smoker	1.7
GWB005687	13.5	37	Female	225	Smoker	1.1
GWB005688	12.7	49	Female	237	Smoker	1.4
GWB005689	12.2	29	Female	137	Non-Smoker	1.4
GWB005692	9.6	41	Male	290	Non-Smoker	2
GWB005691	10.2	36	Female	165	Non-Smoker	1.8
GWB005678	11.3	46	Male	415	Non-Smoker	1.6
Mean	11.4	41.3		242.1		1.5
Standard Deviation	1.22	10.2		66.4		0.2
SEM	0.27	2.3		14.9		0.05

Table 2. Characteristics of Nondiabetic Patients in the Isopropyl Nitrite Studies

Sample ID	HbA1C (%)	Age (yrs)	Gender	Weight (lbs)	Smoker status	Oxidation Time (min)
GWB005538	5.2	25	Male	166	Non-Smoker	4.5
GWB005539	5.4	28	Male	279	Smoker	4.2
GWB005546	5.7	48	Female	167	Non-Smoker	4
GWB005552	5.8	51	Male	253	Non-Smoker	4.1
GWB005554	5.9	42	Male	250	Non-Smoker	4
GWB005555	5.6	54	Male	270	Non-Smoker	4
GWB005558	5.8	65	Male	273	Non-Smoker	5.3
GWB005565	5.3	37	Male	190	Smoker	5.7
GWB005566	5.8	61	Male	174	Non-Smoker	3.8
GWB005570	5.4	47	Male	194	Non-Smoker	4.2
GWB005698	6	51	Male	395	Smoker	5.1
GWB005697	4.8	31	Female	138	Smoker	5.8
GWB005681	5.6	43	Male	155	Smoker	4.4
GWB005676	4.9	42	Female	196	Smoker	5.2
GWB005677	5.7	54	Male	210	Non-Smoker	4.5
GWB005680	5.6	57	Male	198	Non-Smoker	4.4
GWB005699	5.3	19	Male	141	Non-Smoker	4.7
GWB005702	5.9	46	Male	265	Non-Smoker	4.9
GWB005704	5.3	64	Male	212	Non-Smoker	4.3
GRP000480	4.7	39	Female	163	Non-Smoker	5
Mean	5.5	45.2		214.5		4.6
Standard Deviation	0.37	12.4		60.8		0.57
SEM	0.08	2.8		13.6		0.13

A laboratory spectrophotometer equipped with a strip chart recorder was employed to monitor the formation of methemoglobin at 436 nm. A small table top centrifuge was used to separate plasma from the red blood cells. To determine the oxidation time's blood samples were centrifuged for 2000g for 20 min to remove any remaining plasma. The remaining packed Red Blood Cells (RBCs) were aerated and washed in 20 mM Phosphate Buffer Saline (PBS) at pH 7.2 followed by another centrifugation to remove the saline. This procedure of centrifugation, aeration and washing was repeated. The RBCs were then resuspended in 20 mM PBS (pH 7.2) for a maximum of 60 min prior to testing. A 0.01 mL portion of resuspended RBCs was hemolyzed by the addition of 1.0 mL of distilled water and adjusted to a final volume of 2.6 mL by the addition of 20 mM PBS (pH 7.2). The hemoglobin solutions were then adjusted to a standard absorbance (e.g., A = 1.0 ± 0.2) at a wavelength of 436nm with more 20 mM PBS (pH 7.2). The 2.6 mL aliquot of this hemoglobin solution was then added to a 0.05 mL aliquot of 0.1% isopropyl nitrite in ethanol solution. A final concentration of 215 µmol/L was obtained after its addition to the hemoglobin solution. In both studies the above gave a final hemoglobin concentration between 6 and 9 µmol/L. All of the above solutions were then placed in cuvettes and the reaction measured in a spectrophotometer equipped with a chart recorder set at a wavelength of 436 nm. This is a suitable wavelength for measuring and distinguishing oxyhemoglobin and methemoglobin. The spectrophotometer chart recorder then generated graphic representations of the conversion of oxyhemoglobin into methemoglobin as a function of time. The terminal period or asymptotic phase corresponds to essentially 100% methemoglobin formation. The final absorbance was found to be approximately A = 0.5 ± 0.1. All hemoglobin oxidation times (in min) obtained have been included in Tables 1-2 for these samples. According to Colton the appropriate test to use for these data is the Student's t-test for independent samples. The data were analyzed using an Excel spreadsheet on a Microsoft computer. The significance level has been considered to be $P < 0.05$ [20-21].

Results

Diabetic samples all had HbA1C values >6.5% as is presented in Table 1. The normal samples as presented in Table 2 were assessed as nondiabetic because HbA1C values of <6.5% were obtained. Student's t-test for independent samples has revealed that the HbA1C values likely to belong to totally different populations, i.e., diabetic vs. nondiabetic, because we can now reject the null hypothesis. For the isopropyl nitrite studies the findings of the HbA1C percentages revealed that the Diabetes blood mean ± standard error of the mean (SEM) was 11.4 ± 0.27%, while that of the non diabetics blood had a mean ± SEM of 5.5 ± 0.08%. Thus, the percentage differences between the two populations was statistically significant ($P<0.05$), and this means that these two populations are good groups on which to undertake the alkyl nitrite oxidation studies as is shown in the column comparison of the means ± SEM in Figure 1. For isopropyl nitrite the mean oxidation time of the Diabetes blood ± SEM was 1.5 ± 0.05 min whereas the mean oxidation times of the non-diabetics blood ± SEM was 4.6 ± 0.13 min as shown in the column comparison of the mean ± SEM in Figure 2. Based on an independent Student's t-test, the time taken for diabetics erythrocytes to undergo oxidation was significantly shorter ($P<0.05$) than the no diabetic controls.

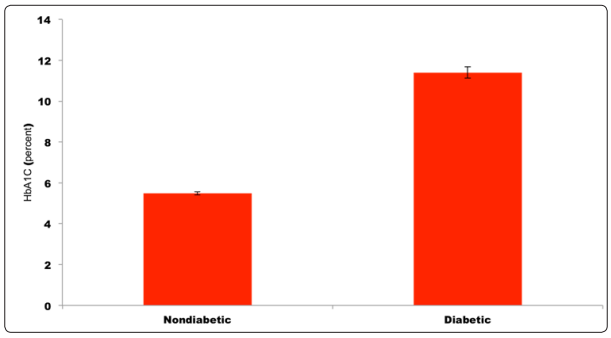

Figure 1: Column Comparison of Means for the Percent Hba1c of the Hemoglobin of Diabetics and Non Diabetics Blood Used In the Isopropyl Nitrite Studies

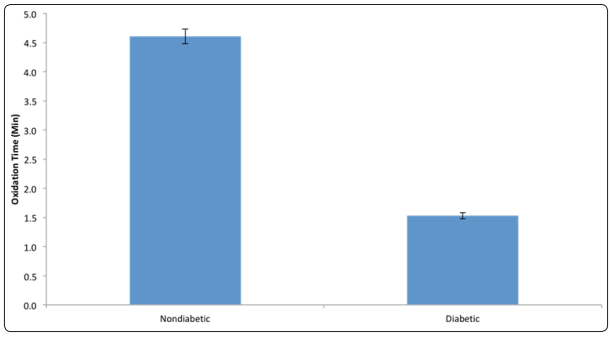

Figure 2: Column Comparison of Means for the Oxidation Times of the Hemoglobin of Diabetics and Non-Diabetics Blood by Isopropyl Nitrite

The mean weight ± SEM of the diabetic patients in this study was 242.1 ± 14.9 with a standard deviation of 66.4, while nondiabetics mean weight was 214.5 ± 13.6 with a standard deviation of 60.8. Thus the difference in the mean weights of the diabetics vs. nondiabetics is not statistically significant (P>0.05). Likewise, the ages of the diabetics vs. nondiabetics is not statistically significant (P>0.05), i.e., 41.3 ± 2.3 (σ = 10.2) vs. 45.2 ± 2.8 (σ = 12.4). Thus, these aforementioned variables did not appear to influence the above findings. Also the ratios of non-smokers to smokers were quite similar (15:5 for diabetics vs. 14:6 for nondiabetics) meaning that the two groups were well matched. Thus, these aforementioned variables did not appear to influence the above findings.

Discussion

Interestingly, the enhanced susceptibility to isopropyl nitrite induced oxidation reactions occurred in Type 2 diabetics blood illustrates that HbA1C oxidation to methemoglobin is a direct function of the amount of HbA1C present as opposed to metabolic differences in the type 1 and type 2 diabetes, i.e., a clear inverse relation appears to exist between the amount of HbA1C present and the oxidation time. Hence, any untreated diabetic simply has a greater percentage of HbA1C than a non-diabetic, e.g. 11.4% vs. 5.5%, as is shown in Figure 1 [22].

Mousse found that Methemoglobin formation by autoxidation was significantly higher from HbA1C than from the non Glycated HbA0. After the glycosylation of HbA0 occurs the end product of this irreversible rearrangement reaction yields a permanently altered molecule known as HbA1C which has been reported to be a less stable molecule than HbA0. Compared to HbA0, HbA1C undergoes faster autoxidation. In addition Glycation of hemoglobin results in modifications of this molecule. Namely, the alpha helix content is reduced and more tryptophan residues become accessible to the surface. This results in increased thermolability as well as weakened heme globin linkages in HbA1C vs. HbA0. FTIR spectral analysis revealed elevated HbA1C levels caused secondary structural modifications that led to decreased structural stability in diabetics whose HbA1C was ≥ 9.0%. These changes are reported to lead to enhanced oxidative stress in diabetics [23-25]. Thus, these preliminary findings with isopropyl nitrite indicate that diabetics have hemoglobin that exhibit greater oxidative stress to alkyl nitrite owing to a higher percentage of HbA1C. Moreover, this is reinforced by earlier studies wherein other alkyl nitrites such as ethyl nitrite, butyl nitrite, pentyl and hexyl nitrite all gave equivalent results with Type 2 diabetics blood [1-6]. These similar findings could be attributed to the fact that these nitrite esters differ only in the type of alkyl group present. Whether there are two, three, four, five or six saturated carbons attached to the esters oxygen makes no difference in terms of these compound's ability to induce enhanced Methemoglobin formation in Type 2 diabetics blood.

Conclusion

The present study shows a statistically significant enhanced hemoglobin oxidation time for type 2 Diabetes blood. This relationship between methemoglobinization time with HbA1C values for diabetics blood vs. normal blood using Student's t-test for independent samples was found to be statistically significant (P<0.05). This implies that Methemoglobinization is an important indicator for oxidative stress in diabetic's blood and makes any use of alkyl nitrites to be avoided by diabetics since their blood much more easily undergoes conversion from oxyhemoglobin to methemoglobin than with nondiabetics. The strength of these findings are that they apply to human blood samples of diabetics and normal individuals, i.e., the in vitro findings are clear cut. Nevertheless, this does not necessarily prove these findings would be the same in vivo.

Acknowledgements

The author wishes to acknowledge Ms.Virginija Kirkiliene the Multiuse Laboratory Supervisor of the Los Angeles campus of National University for her technical assistance in helping to prepare this publication.

References

1. Tarburton J (2013) Amyl nitrite induced hemoglobin oxidation studies in diabetics and non-diabetics blood. J Diabetes and Metabolism 4: 258-260.
2. Tarburton JP (2014) Hemoglobin oxidation studies in diabetics blood comparing the effect of sodium nitrite vs. amyl nitrite. Diabetes Research and Treatment 1: 112-117.
3. Tarburton JP (2015) Hemoglobin oxidation studies in diabetics blood and normal blood: The effects of butyl nitrite and pentyl nitrite. Diabetes Research and Treatment 2: 127-137.
4. Tarburton JP (2016) Linear regression analysis and correlation findings in hemoglobin oxidation studies in diabetics blood and normal blood: The effects of butyl nitrite and pentyl nitrite. Diabetes Research and Treatment 3: 134-142.
5. Tarburton JP (2017) Comparative studies on the effects of ethyl nitrite and hexyl nitrite Induced hemoglobin oxidation of diabetics blood and normal blood. ARC Journal of Diabetes and Endocrinology 3: 1-5.
6. Tarburton JP (2019) Methemoglobinization of diabetics blood with ethyl nitrite and hexyl nitrite. Journal of Clinical Endocrinology 1: 24-28.
7. Dan Longo, Anthony Fauci, Dennis Kasper, Stephen Hauser, J. Jameson, et al. (2012) "Harrison's Principal's of Internal Medicine". 18th ed.. McGraw Hill, NY Vol 1: 857-858

http://onemansblog.com/wp-content/uploads/2016/06/Harrisons%20Manual%20of%20Medicine,%2018th%20Edition.pdf.

8. Kiese M (1974) "Methemoglobinemia: a Comprehensive Treatise: Causes, Consequences, and Correction of Increased Contents of Ferrihemoglobin in Blood." CRC Press 259p.
9. Beck F, Guignard R, Richard JB (2014) Poppers at top: Alkyl nitrites use in France. Medicine Sciences (Paris) 30: 916-921.
10. Lusher A (2016) So what exactly are poppers? The Independent. https://www.independent.co.uk/news/uk/so-whatexactly- are-poppers-a7052141.html.
11. Romanelli F, Smith KM, Thornton AC, Pomeroy C (2004) Poppers: Epidemiology and Management of inhaled nitrite abuse. Pharmacotherapy 24: 69-78.
12. Davies AJ, Kelly SP, Bhatt PR (2012) 'Poppers maculopathy'--an emerging ophthalmic reaction to recreational substance abuse. Eye (correspondence) 26: 1479-86.
13. Rewbury R, Hughes E, Purbrick R, Prior S, Baron M (2017) Poppers: Legal highs with questionable contents? A case series of poppers maculopathy [Abstract]. Brititsh Journal of Ophthalmology 101: 1530-1534.
14. Davies AJ, Borschmann R, Kelly SP, Ramsey J, Ferris J, et al. (2017) The prevalence of visual symptoms in poppers use: a global survey. BMJ Open Ophthalmol 1: e000015.
15. Cha HJ, Kim YJ, Jeon SY, Kim YH, Shin J et al. (2016) Neurotoxicity induced by alkyl nitrites: Impairment in learning/memory and motor coordination. [Abstract] Neuroscience Letters 619: 79-85.
16. Bernasconi B, Konrad CJ, Fischer S (2014) Case Report-A dangerous intoxication after ingestion of alkyl nitrite ('poppers'). Anästhesiol. Intensivmed Notfallmed Schmerzther 49: 654-8.
17. Hall A, Stessel B, Bergmans D, Schnabel R (2012) Two cases of acquired methemoglobinemia. Acta Anaesth Belg 63: 97-100.
18. Wong A, Koutsogiannis Z, Greene S, McIntyre S (2013) A case of hemolysis and methemoglobinemia following amyl nitrite use in an individual with G6PD deficiency. Journal of Acute Medicine 3: 23-25.
19. National Diabetes Fact Sheet (2011) U.S. Department of Health and Human Services, Centers for Disease Control and Prevention https://www.cdc.gov/diabetes/pubs/pdf/methods11.pdf.
20. Weissbluth M (1974) Hemoglobin: Cooperativity and electronic properties. In: Kleinzellar A, Springer GF, Whitman HG (eds), Molecular Biology, Biochemistry and Biophysics, Sringer-Verlag, New York Vol 15: 136-141.
21. Colton T (1974) Statistics in Medicine. Little, Brown and Co. Boston, MA pp99-150 and 207-211.
22. Moussa SA (2018) Oxidative stress in diabetes mellitus. Romanian Journal of Biophysics 18: 225-236.
23. Moussa SA (2007) Biophysical changes in red blood cells and hemoglobin components of diabetic patients. Journal of Genetic Engineering and Biotechnology 1768: 27-32.
24. Sen S, Kar M, Roy A, Chakraborti AS (2005) Effect of nonenzymatic glycation on structural and functional properties of hemoglobin. Biophys Chem 113: 289-98.
25. 25.Ye S, Ruan P, Yong J, Shen H, Liao Z, et al. (2016) The impact of the HbA1c level of type 2 diabetics on the structure of haemoglobin. Sci Rep 6: 33352.